ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Swietenia macrophylla King Wood Residues: A Source of Cycloartane Triterpenoids.

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ABSTRACT

Cycloartanes are tetracyclic triterpenoids that contain the cyclopropane ring at C-9 and C-10. They widely occur in plants and are renowned for their pharmacological properties. In the Meliaceae family, these triterpenoids have been found in some genera; however, they have been rarely reported in *Swietenia*. In the present work, we identified for the first time cycloartane triterpenes in wood residues of *Swietenia macrophylla* (mahogany) and evaluated their activity against fungi of medical interest. Samples of mahogany from windows with more than 30 years of use were donated to this study and then subjected to extractions with hexane followed by methanol. The hexane extract was subjected to chromatographic procedures for purification of cycloeucalenol and ß-sitosterol esterified (mixture), cycloeucalenone and cycloeucalenol. Chromatographic fractionation of fraction 1 of the methanolic extract led to the purification of cycloeucalenol, cycloart-23*E*-en-3β,25-diol and a mixture of 24-metileno-cicloartan-3β-ol and cycloartenol. In the antifungal assay, the most active compounds were a mixture of cycloeucalenol and ß-sitosterol with a MIC of 80 µg/mL for *Candida albi*cans and *Cryptococcus gattii*; cycloeucalenol with a MIC of 80 µg/mL for *C. gattii*. This study was an opportunity to add to the knowledge on the secondary metabolites of wood of this species as well as search for active principles.

Keywords: Mahogany, Meliaceae, NMR, antifungal

https://doi.org/10.33887/rjpbcs/2023.14.1.1

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ISSN: 0975-8585

INTRODUCTION

Cycloartanes are tetracyclic triterpenoids that contain the cyclopropane ring at C-9 and C-10 and occur frequently in plants. Over the last few decades, many studies related to the pharmacological properties of these compounds have reported their antidiabetic [1, 2], leishmanicidal [3], and antitumor effects, among others [4]. However, there are few reports of antifungal assays with cycloartane triterpenes. Ekhuemelo et al. [5] showed the antifungal potential of this type of triterpene for soft rot, wet rot and white rot of wood, as well as for other diseases caused by wood fungi. In the Meliaceae family, the cycloartane-type triterpenoids have been found in the genera *Aglaia* [6, 7], *Trichilia* [8-10], *Guarea* [11, 12], and *Toona* [13]; however, these have been rarely reported in the genus *Swietenia*.

Swietenia macrophylla King, known as Brazilian mahogany, is a robust tree that dominates the forest canopy and is a valuable timber species that has been exploited in a predatory way in the Amazon. The overexploitation and destruction of its habitat have threatened mahogany populations throughout its area of distribution, and have led to the inclusion of the species in Appendix II of CITES (Convention for International Trade in Endangered Species of the Wild Fauna and Flora), which reflects the international concern about the future of the species [14]. Phytochemical studies of the species indicate the predominance of limonoids [15-20], which have a wide variety of biological activities.

In the present work, we identified for the first time cycloartane triterpenes in wood residues of *S. macrophylla* and evaluated their activity against fungi of medical interest.

MATERIALS AND METHODS

General experimental procedures

NMR spectra were measured in a spectrometer Bruker Fourier-300 with TMS as the internal standard. LC-HRMS measurements were obtained using a mass spectrometer (MicroTOF-QII, Bruker Daltonics) connected to a liquid chromotagraph (Prominence UFLC Shimadzu). Chromatographic separations were carried out on silica gel 60 (Merck), microcrystalline cellulose (Merck), florisil (Sigma-Aldrich) and Sephadex LH-20 (Sigma- Aldrich). Analytical and preparative TLC were carried out on pre-coated silica gel 60 F254 plates (Merck).

Woody residues and extracts

Samples of mahogany from windows with about 30 years of use in a public building were donated to the Wood Technology Laboratory of the National Institute for Amazonian Research (INPA) where they were submitted to studies of their technological properties as well as confirmation of the species as *S. macrophylla* based on sensory and anatomical characteristics and comparison with standard xylotheque samples. The wood residues generated in these activities were submitted to phytochemical analysis. Thus, the wood residues (863.8 g) were ground in a Wiley mill, and then subjected to extractions with hexane followed by methanol (seven days in each solvent), which after evaporation under reduced pressure gave a hexane extract (5.4 g) and a methanolic extract (13.05 g).

Chromatographic fractionation of extracts of *S. macrophylla*

The hexane extract, fractionated over silica gel in the column (70-230 mesh; h x Φ = 39.6 x 2.3 cm) and eluted with hexane, hex-EtOAc (2-30%), yielded twenty-two fractions. The grouped fractions 6-8 (459.9 mg), 9-10 (270.5 mg) and 15-16 (448.2 mg) and fraction 18 **(**152.6 mg) were subjected to new chromatographic procedures. For the chromatographic fractionation of fractions 6-8, a column of silica gel (230-400 mesh; h x Φ = 31.7 x 2.8 cm) was used, which was eluted with hexane, hex-EtOAc (2-40%), and then subfraction 7 was subjected to separation via preparative TLC (20 x 20 cm) on silica gel 60 F254 eluted with hex:acetone:CH_2Cl_2 (95:3:2 for purification of compounds **1** and **2** (15.5 mg) and **3** (2.6 mg). Fractions 9-10 were subjected to a cellulose column (h x Ø = 28.6 x 2.9 cm), eluted with hexane, which gave mixture **4a and 4b** (8.8 mg). The treatment of fractions 15-16 with hot MeOH resulted in the purification of compound **5** (448.2 mg). Fraction 18 showed a predominance of the mixture of ß-sitosterol and stigmasterol.

The fractionation of the methanolic extract in the silica gel column (70-230 mesh; h x Ø = 42.7 x

4.5 cm), which was eluted with hexane, hex-MeOH (2-40%), resulted in thirty-eight fractions. Fraction 1 (169.9 mg) fractionated in the silica gel column (230-400 mesh; h x Ø = 46.3 x 2.4 cm), which was eluted with hex:AcOEt (2-40%) resulted in the isolation of compounds **5** (51.9 mg), **6** and **7** (9.3 mg), and **8** (1.1 mg).

Spectroscopic data of compounds

Cycloeucalenol ester (**1**): White solid. ^{1}H NMR (300 MHz, $CDCl_3$) (multiplicity, J/Hz): 4.72 (sl, H-24'a), 4.67 (d, J =1.2 Hz, H-24'b), 4.53 ddd (ddd, J = 16.3, 10.3 and 4.6 Hz , H-3), 2.30 (t, J = 7.6 Hz, H-2'), 2.24 (sept, H-25), 1.61 (m, H-3'), 1.30-1.28 [m, (CH_2)n], 1.05 (d, J=6.8 Hz, H-27), 1.04 (d, J=6.8 Hz, H-26), 1.03 (s, H-28), 0.97 (s, H-18), 0.91 (d, J =.6.9 Hz, H-21), 0.89 (t , J = 6.9 Hz, CH_2**CH_3**), 0.85 (d, J = 6.6 Hz, H-29***),*** 0.41 (d, J =.4.0 Hz, H-19a), 0.16 (d, J = 4.0 Hz, H-19b***).*** ^{13}C NMR (75 MHz, $CDCl_3$): Table 1.

Cycloeucalenone (**3**): White solid. ESIMS m/z m/z 425.43 $[M+H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) (multiplicity, J/Hz): 4.72 (sl, H-24'a), 4.67 (d, J = 1.3 Hz, H-24'b), 2.24 (sept, H-25), 1.05 (d, J = 6.8 Hz, H-26 and H-27), 1.01 (s, H-18), 1.01 (d, J =6.7, H-29). 0.92 (d, J = 6.2 Hz, H-21), 0.92 (s, H-28), 0.63 (d, J = 4.0 Hz, H-19a), 0.41 (d, J = 4.0 Hz, H-19b).^{13}C NMR (75 MHz, $CDCl_3$): Table 1.

Cycloeucalenol **(5**): White solid. ESIMS m/z 427.50 $[M+H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) (multiplicity, J/Hz): 4.72 (sl, H-24'a), 4.67 (d, J = 1.3 Hz, H-24'b), 3.22 (ddd, J = 10.6, 9.1, 4.5 Hz H-3), 2.24 (sept, H-25), 1.05 (d, J = 6.8 Hz, H-26), 1.04 (d, J = 6.8 Hz, H-27), 1.01 (d, J = 6.3 Hz, H-29). 0.98 (s, H-18), 0.91 (d, J = 6.3 Hz, H-21), 0.90 (s, H-28), 0.39 (d, J = 4.0 Hz, H-19a), 0.16 (d, J = 4.0 Hz, H-19b). ^{13}C NMR (75 MHz, $CDCl_3$): Table 1. 24-metileno-cicloartan-3β-ol (**6**): White solid. ESIMS m/z 441.44 $[M+H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) (multiplicity, J/Hz): 4.72 (sl, H-24'a), 4.67 (d, J = 1.2 Hz, H-24'b), 3. 32 (dd, J = 10.7 and 4.2 Hz, H-3), 2.24 (t, J = 6.9 Hz, H-25), 1.05 (d, J = 6.8 Hz, H-26), 1.02 (d, J = 6.8 Hz, H-27), 0.97 (s, H-18 and H-30), 0.90 (d, J = 6.27 Hz H-21), 0.89 (s, H-28); 0.81 (s, H-29), 0.56 (d, J = 4.1 Hz, H-19a), 0.35 (d, J = 4.1 Hz, H-19b). ^{13}C NMR (75 MHz, $CDCl_3$): Table 1.

Cycloartenol (**7**): White solid. ESIMS m/z 427.37 $[M+H]^+$. ^{1}H NMR (300 MHz, $CDCl_3$) (multiplicity, J/Hz): 4.72 (t, J = 6.6 Hz, H-24), 3.32 (dd, J = 10.7 and 4.2 Hz, H-3), 1.69 (s, H-27), 1.61 (s, H-26), 0.97 (s, H-18 and H-30), 0.90 (d, J = 6.27 Hz, H-21), 0.89 (s, H-28). 0.81 (s, H-29), 0.56 (d, J = 4.1 Hz, H-19a), 0.35 (d, J = 4.1 Hz, H-19b). ^{13}C NMR (75 MHz, $CDCl_3$): Table 1.

Cycloart-23*E*-en-3β,25-diol (**8**): White solid. ^{1}H NMR (300 MHz, CD_3OD). (multiplicity, J/Hz): 5.59 (m, H-23 and H-24), 3.25 (dd, J = 11.2 and 5.0 Hz, H-3), 1.27 (s, H-26 and H-27), 1.02 (s, H-18), 0.95 (s, H-30), 0.94 (s, H-28), 0.91 (d, J = 6.4 Hz, H-21), 0.81 (s, H-29), 0.58 (d, J = 3.7 Hz, H-19a), 0.37 (d, J = 3.7 Hz, H-19b). ^{13}C NMR (75 MHz, CD_3OD): Table 1.

Antifungal assay

Antifungal susceptibility was performed using *Candida albicans* (ATCC 60193)*, Candida Krusei (*ATCC 34135) and *Cryptococcus gattii* (ATCC 32269) obtained from the culture collection at the National Institute of Amazonian Research (INPA). Minimum inhibitory concentration (MIC) assays were performed using the broth microdilution method, as described by the Clinical and Laboratory Standards Institute [21]. Fluconazole was used as the antifungal standards.

RESULTS AND DISCUSSION

The hexane extract from the wood residues of *S. macrophylla* provided triterpenes of the cycloartane type (**1**, **3** and **5**) and esterified steroid (**2**), in addition to a mixture of the triterpenes ursane and oleanane (**4a**,**b**). Chromatographic fractionation of fraction 1 of the methanolic extract led to the purification of cycloartane triterpenes (**5**-**8**), in addition to the mixture of ß-sitosterol and stigmasterol.

The ^{1}H NMR spectra showed doublets with coupling constants at 4.0 Hz, cyclopropane characteristic of norcycloartane in δ 0.63–039 (H-19a), 0.41–0.16 (H-19b) of triterpenes **1**, **3** and **5;** δ 0.58–0.56 (H-19a), and 0.37–0.35 (H-19b) of cycloartane triterpenes **6-8**. The absence of one of the methyl groups at C-4 was detected from the spectra of ^{1}H NMR of **1**, **3** and **5**, then the other methyl attached to carbon 4 was detected as a doublet at δ 0.85 (6.6 Hz of triterpene **1**), 1.01 (6.7 Hz of triterpene **3**) and 1.01 (6.3 Hz of triterpene **5**). The signals that characterized the triterpene side chains were δ 4.72 (sl, H-24'a)

ISSN: 0975-8585

and 4.67 (d, J = 1.2 0u 1.3 Hz, H-24'b) of terminal methylene attached to C-24 of triterpenes **1**, **3**, **5** and **6**. The triplet at 4.72 (J = 6.6 Hz) characterized the double bond in H-24 of triterpene **7**, and the multiplet at δ 5.59 (H-23 and H-24) is characteristic double bond in H-23 of **8.** The carbon signal shifts of these triterpenes are shown in Table 1**.**

Table 1: ^{13}C NMR of cycloartane triterpenes from wood residues of *S. macrophylla*

N0	1	3	5	6	7	8
1	30.48	32.8	30.7	31.9	31.9	31.8
2	30.98	41.0	34.8	30.3	30.3	29.6
3	78.40	213.5	76.5	78.8	78.8	78.0
4	41.51	50.0	44.5	40.4	40.4	40.1
5	43.44	46.0	43.3	47.0	47.0	47.2
6	24.68	25.2	24.6	21.1	21.1	20.8
7	25.19	28.2	25.1	26.0	26.0	27.6
8	46.93	47.1	46.8	48.0	48.0	48.0
9	23.62	24.9	23.5	19.9	19.9	19.7
10	29.48	29.2	29.5	26.4	26.4	25.9
11	26.96	25.8	26.9	26.0	26.0	26.0
12	32.82	32.7	32.8	32.8	32.8	32.6
13	45.32	45.3	45.3	45.2	45.2	45.0
14	48.88	48.7	48.8	48.8	48.8	48.5
15	34.83	35.4	34.9	34.9	34.9	35.3
16	28.11	27.1	28.1	28.1	28.1	25.8
17	52.17	52.2	52.2	52.2	52.2	51.7
18	17.82	17.9	17.8	18.0	18.0	17.2
19	27.20	27.0	27.2	29.9	29.9	29.5
20	36.13	36.1	36.1	36.1	35.8	36.3
21	18.33	18.3	18.3	18.3	18.3	17.4
22	35.34	34.9	35.3	35.5	36.3	38.7
23	31.30	31.2	31.3	31.3	24.9	124.6
24	156.9	156.9	156.9	156.9	125.2	139.2
24'	105.91	105.9	105.9	105.9		
25	33.79	33.8	33.7	33.7	130.9	69.7
26	21.87	22.0	22.0	21.87	17.65	28.6
27	22.00	21.8	21.8	22.0	25.7	28.7
28	19.14	19.1	19.1	19.3	19.3	18.3
29	14.41	10.7	14.4	14.0	14.0	13.3
30				25.4	25.4	24.6
1'	173.7					
2'	34.96					
3'	25.07					
$(CH_2)n$	29.6-29.1					
$\underline{CH_2}CH_3$	22.71					
$CH_2\underline{CH_3}$	14.14					

Thus, based on ^{1}H, ^{13}C NMR data combined with 2D spectra (HSQC and HMBC), the triterpenes were identified as cycloeucalenol ester (**1**), cycloeucalenone (**3**), cycloeucalenol (**5**), 24-methylenecycloartanol (**6**), cycloartenol (**7**) and cycloart-23E-eno-3β,25-diol (**8**). Cycloartenol and 24-methylenecycloartanol were also isolated together by Nair et al. (2020), and the data of the NMR were similar to compounds **6** and **7**. Cycloeucalenol (**5**) was the main compound in *S. macrophylla* residues, indicating that it is responsible for wood resistance, as previous studies have shown that this triterpene possesses antifungal activity and could be used in the control of the soft-rot, brown-rot, wet rot and white-rot of wood and other diseases caused by wood fungi (Ekhuemelo et al. 2019).

The characteristic signals of the mixture of α-amyrin (**4a**) and β-amyrin (**4b**) were verified in the ^{1}H NMR spectrum due to the olefinic hydrogens at δ 5.13 t (J = 3.5 Hz, **4a**) and 5.19 t (J = 3.5 Hz , **4b**), in

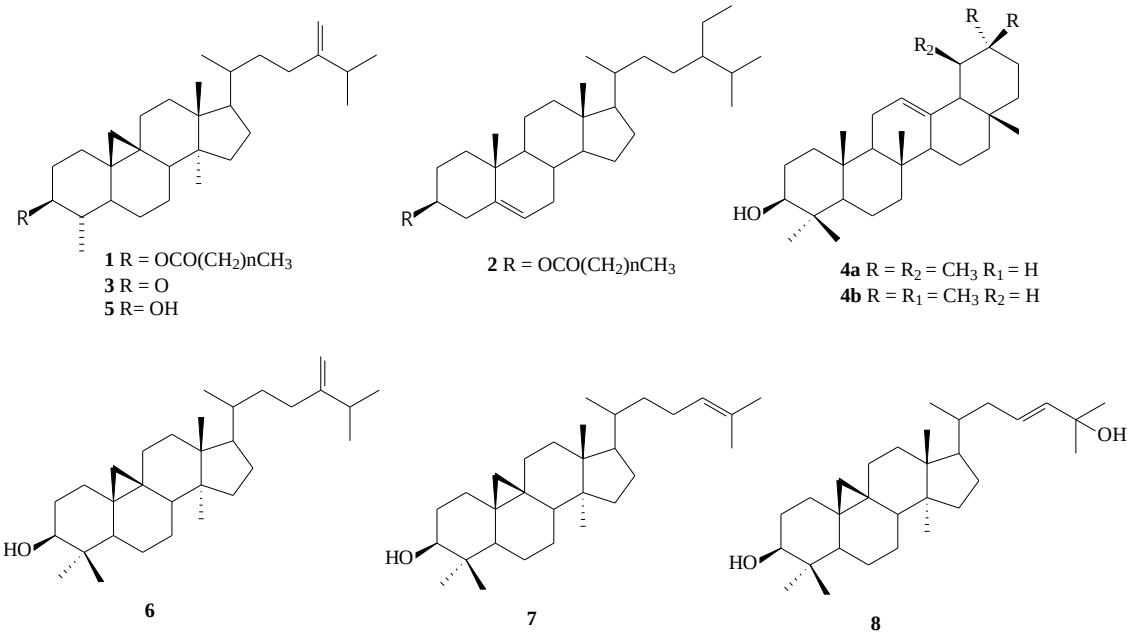
ISSN: 0975-8585

addition to the carbinolic hydrogens at δ 3.22 dd (J =10.4 and 5.0 Hz, **4a,b**). The ^{13}C NMR spectrum showed the signals of olefinic carbons at δ 124.40 and 139.57 (**4a**), δ 121.70 and 145.20 (**4b**), carbinolic carbon at δ 79.06 and 79.04, respectively. Compound **2** was identified as ß-sitosterol esterified at C-3.

The cycloartane triterpene 24-methylenecycloartanol (**6**) was previously identified in *Swietenia macrophylla* bark [22], and the other compounds identified in this work have their first previous records in this species.

In the antifungal assay the most active compounds were **1** and **2** (mixture of cycloeucalenol and ß-sitosterol) with MIC of 80 µg/mL for *Candida albi*cans and *Cryptococcus gattii*; cycloeucalenol (**5**) with a MIC of 80 µg/mL for *C. Gattii* and 160 µg/mL for *C. albicans*. The mixture of tritepenes **6** and **7** did not show activity for the three fungal species. No activity was found for *Candida krusei*.

Figure 1: Structures of the cycloartane triterpenes from *S. macrophylla*



CONCLUSION

Phytochemical study carried out with *Swietenia macrophylla* showed that wood residues proved to be a source of triterpenes mainly of the cycloartane types with an additional carbon on C-24, C-24 and C-25 double bond, C-23 and C-24 double bond, norcycloartane with an additional carbon on C-24. This search was an opportunity to add knowledge to the secondary metabolites of wood of this species as well as in the search for active principles

ACKNOWLEDGMENTS

The authors are grateful for the support from the Fundação de Amparo à Pesquisa Estado do Amazonas (FAPEAM), Grant No 01.02.016301.03412/2021-78, and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) for the scholarship granted to Henrique Cativo dos Santos.

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